Expansion of Basic Biobricks Into Useful Part Families

By The iGEM Team at UC Davis

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Electrical engineer wants to build a SHO.
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Diagram the circuit.
Electrical engineer wants to build a SHO.

Diagram the circuit.
Develop a mathematical model.

\[
\frac{d^2i(t)}{dt^2} + \frac{R}{L} \frac{di(t)}{dt} + \frac{1}{LC} i(t) = 0
\]
Electrical engineer wants to build a SHO.

Diagram the circuit.
Develop a mathematical model.
Define component parameters.
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Find parts with desired parameters.
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- Find parts with desired parameters.
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Motivation

Parts Registry is meant to allow synthetic biologists to find the parts they need.

Large selection of both basic and composite parts.

Part families: Groups of parts which are mutually derived from one basic part.

Share a common functionality but have different parameters.

Example: Constitutive Promoter Library
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Example: Constitutive Promoter Library
The creation of new part families improves the usefulness of the Registry.

We set out to develop a process by which basic BioBricks could be expanded into part families. This process would:

- Generate mutant family-members that fill a parameter space with a resolution appropriate for a given circuit.
- Thoroughly characterize these mutants.
- Be accessible enough to encourage other teams to create their own part families.
Making Part Families

1. Target Part
   - Basic Part
     - Promoters
     - Reporters
     - Regulatory Proteins
     - Mutagenic PCR
     - Mutator E. coli
     - DNA shuffling
     - Site-directed mutagenesis
     - Visual
     - Cell Death/Survival
     - Plate Reader
     - FACS
     - Plate Reader
     - Flow Cytometry
2. Mutagenesis
   - Random
   - Targetted
     - Manual
     - Automatic
3. Screening
4. Characterization
Making Part Families

1. Target Part

2. Mutagenesis

3. Screening

4. Characterization

Basic Part

Promoters
Reporters
Regulatory Proteins

Mutagenic PCR
Mutator E. coli
DNA shuffling

Site-directed mutagenesis
Visual
Cell Death/Survival
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Manual
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Making Part Families

1. Target Part → Basic Part
   - Promoters
   - Reporters
   - Regulatory Proteins

2. Mutagenesis

3. Screening

4. Characterization
Integral to all circuits

Easy to screen

Repressible promoters can be “tuned” with repressors
  • LacI can be “tuned” by IPTG

Good Model:
  • J23101 and associated family
  • Many variants with different activities
• GFP is a model reporter
  • Average length
• Useful for prototyping mutagenesis process
• Can help estimate mutation rate
  • Small DNA changes can alter color
  • Screenable with the naked eye or under UV lamp
Making Part Families

1. Target Part
2. Mutagenesis
   - Mutagenic PCR
   - Mutator E. coli
   - DNA shuffling
3. Screening
   - Random
   - Targetted
4. Characterization
   - Site-directed mutagenesis
• Mutagenic PCR (Error-prone PCR)
  • Well known
  • Universal
    - Works with any BioBrick using VF2 and VR primers
  • We optimized it to make 1-7bp mutations per 1KB
  • Can run consecutive reactions for more mutations
Making Part Families

1. Target Part

2. Mutagenesis

3. Screening

4. Characterization

- GFP
- LacI

- Mutagenic PCR

- Manual
- Automatic

- Visual
- Cell Death/Survival
- Plate Reader
- FACS
Quickly confirms mutagenesis
- Direct
  - GFP production linked to promoter activity
  - Can make a good guess at “strong” and “weak” mutants
- Simple
• Fast
• Low error
• Data
  • Scripts can sort data for mutant selection
• Automated
Making Part Families

1. Target Part
   - GFP
   - LacI
2. Mutagenesis
   - Mutagenic PCR
3. Screening
   - Visual Plate Reader
4. Characterization
   - Plate Reader
   - Flow Cytometry
• Easy to transfer from screen
  • Still simple, fast, has minimal error
• High throughput
• Automated
  • Put plate in at night, get data in the morning
  • One mutant per plate
  • ~12 hours per run
  • 2 mutants per day
Making Part Families

1. Target Part
   - GFP
   - LacI

2. Mutagenesis
   - Mutagenic PCR

3. Screening
   - Visual
   - Plate Reader

4. Characterization
   - Plate Reader
Create a variant family for the LacI repressible promoter (BBa_R0010).

1. Target Part
2. Mutagenesis
3. Screening
4. Characterization
Mutate: 3 rounds of mutagenic PCR

We also generated mutant libraries of the TetR and lambda cI promoters which await further characterization.
**Screen:** Three-step process

1. Visually screen mutant promoters that have been ligated in front of GFP.

- Fluorescence and promoter activity are directly related.
- We can use a plate reader to measure fluorescence.
2. 84 colonies are selected for screening in liquid culture.

- Direct inoculation from transformation plate
- Bottom row contains +/- controls
This graph shows 84 promoter mutants compared to wild type. Green bars represent mutants with activity levels that differ from wild-type by at least 1.5 standard deviations.
3. 28 mutants are selected for further screening.

- Mutants are run in triplicate to assess sampling error.
- If error is large, the screen is repeated to ensure reliable selection.
This graph displays sorted data from a 28-mutant screen. Mutants are nearly linearly spaced, indicating good primary selection.
pBAD regulates repressor transcription.
- When AraC is present, pBAD is repressed.
- Increasing arabinose concentration induces pBAD, driving repressor expression.
- Controlling repressor concentration enables characterization of promoter behavior.
LacI Promoter mutants are characterized one at a time.

- One mutant per plate
- Repressor expression increases with arabinose concentration
- IPTG concentration induces the repressor
Calculation of Relative Promoter Units

\[ RPU_\phi = \frac{S_{cell, \phi}^{SS}}{S_{cell, reference}^{SS}} \]

\[ S_{cell, \phi}^{SS} \approx \frac{Fluorescence(\phi) - Fluorescence(0)}{OD(\phi) - OD(0)} \]

\[ OD = \text{Abs}_{600} \propto \text{Cell Number} \]

Use wild-type basal fluorescence as in-vivo reference
Results

Arabinose (wt%)
Results
Results

[Graph showing the relationship between arabinose concentration and relative fluorescence for wild-type and mutant 5 strains. The graph includes markers for the end of LacI coding region (inactive), CAP binding site, -35, -10, and LacI binding site.]
Used an infinite M200 Plate reader
Wanted an attractive and free (preferably open-source) solution for plotting data on the web

2D: Flot.js

3D: ???
Implemented our own 3D plotting library

- Utilizes HTML 5 Canvas
- May support WebGL in the future

Fully Interactive

Transparency

Axis labeling, grids, error bars, highlighting all supported

- Cross-platform (including smartphones)
- Fast
\[
\frac{dA}{dt} = P - K_{\text{deg}} A
\]
\[
\theta_1 = \frac{K_L^{n_A}}{L_A^{n_L} + K_L^{n_L}}
\]
\[
\frac{dL}{dt} = b_{BAD} + \frac{a_{BAD}}{1 + K_{BAD} (\theta_1 A)^{n_{BAD}}} - K_{\text{deg}}^L L
\]
\[
\frac{dG}{dt} = b_{\text{LacI}} + \frac{a_{\text{LacI}}}{1 + K_{\text{LacI}} (\theta_2 L)^{n_{\text{LacI}}}} - K_{\text{deg}}^{\text{GFP}} G
\]
• Fit to model
UC DAVIS

LacI-Repressible Promoter

FEATURES
- SOFT SECTOR FORMAT COMPATIBILITY
- AUTOMATIC TRACK SEEK WITH VERIFICATION
- READ MODE
  Single/Multiple Sector Write with Automatic
  Sector Search or Entire Track Read
  Selectable 128 Byte or Variable Length Sector
- WRITE MODE
  Single/Multiple Sector Write with Automatic
  Sector Search
  Entire Track Write for Diskette Formatting
  PROGRAMMABLE CONTROLS
  Selectable Track-to-Track Stepping Time
  Selectable Head Setting and Head Engage Times
  Selectable Three Phase or Stop and Direction and Head Positioning Motor Controls

APPLICATIONS
- SYSTEM COMPATIBILITY
  Double Buffering of Data 8-Bit Bi-Directional
  Bus for Data, Control and Status
  DMA or Programmed Data Transfers
  All Inputs and Outputs are TTL Compatible

GENERAL DESCRIPTION
The FO1771 is a MOS/LSI device that performs the functions of a Floppy Disk Controller/Formatter.
The device is designed to be included in the disk drive electronics, and contains a flexible interface.
Characterize!

Promoter (lacI regulated)
This part is an inverting regulator sensitive to LacI and CAP.
It contains two protein binding sites. The first binds the CAP protein, which is generally present in E. coli and is associated with cell health and availability of glucose. The second binds LacI protein.

- In the absence of LacI protein and CAP protein, this part promotes transcription.
- In the presence of LacI protein and CAP protein, this part inhibits transcription.
- LacI can be inhibited by IPTG.
- LacI is coded by BBa_C0010

Usage and Biology
This is a direct copy of bases 0365739 through 0365540 of the E. coli K-12 MG1655 genome, Genbank NC_000913 in reverse complement form. It is the natural promoter for the LacZXY operon. It includes the tail end of the LacI gene coding region, but no promoter region for that partial gene.

Sequence and Features

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<th>Subparts</th>
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Sequence:
1. caatacgcc accgctctt caagcgtt ggcgactg ttattgacg gtttctcag cttgaaacg ggcagtgac gcaacctat

### Assembly Compatibility:
10 12 21 23 25

### Parameters
- biology
- control
- IPTG
- lacI
- direction
- Forward
- negative_regulators
- 1
- o_h
- o_i
- positive_regulators

### Twins
- BBA_I7154 Deleted
- BBA_I714076 Deleted
- BBA_K075387 Planning
- BBA_K252002 Available

### Reviews
1 Registry Star

### Categories
- /map/prokaryote/ecoli/sigma70
- /direction/forward
- /chassis/prokaryote/ecoli
- /promoter
- /regulation/negative
- /classic/regulatory/uncategorized
Part: BBa_R0010

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**Variants**

- BBa_17154
- BBa_1714076
- BBa_K075387
- BBa_K252002

**Twins**

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We hope that future teams will use our optimized protocol to generate families of variants.

Well-characterized families should be the norm.
In Summary

We have:

- Optimized and implemented a pipeline for the generation and characterization of regulatory part families
- Suggested a framework for generation of part families of any BioBrick part, provided a screening solution exists
- Proposed solutions to improve the discovery and usage of part families on the parts registry
- Implemented rich web applications for the visualization of scientific data
Thank You!
We would like to thank our advisors and sponsors!

Faculty Advisors:
- Dr. Marc Faciotti
- Dr. Ilias Tagkopoulos

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- Andrew Yao
- Linh Huynh
- Mike Starr
Thank you for attending our presentation!